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Chemical:	**Methomyl**
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

013770

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: September 30, 1999

MEMORANDUM

SUBJECT: ***Methomyl***: Acute and Subchronic Neurotoxicity Studies

FROM: Yung G. Yang, Ph.D. Yung G. Yang 9/30/99
Toxicology Branch
Health Effects Division (7509C)

THROUGH: Alberto Protzel, Ph.D. 9/30/99
Branch Senior Scientist, Toxicology Branch
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TO: Tom Myers
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Health Effects Division (7509C)

DP Barcode: D248061/D253745
Case: 819319
Submission: S546517/S557602
Chemical: Methomyl
Caswell No.: 549C
PC No.: 090301
Registrant: E.I. du Pont de Nemours & Co.

ACTION REQUESTED: Review acute and subchronic neurotoxicity studies in rats for methomyl which were submitted to fulfill data gaps. These studies were submitted as 6a2.

RESPONSE: The acute neurotoxicity study in rats (MRID 44487501) and subchronic

neurotoxicity study in rats (MRID 44666201) have been reviewed and were found to be acceptable/guideline. The Toxicology Branch acknowledged that the new information (inhibition of cholinesterase activity) was submitted as 6a2. The Data Evaluation Records (DERs) will be submitted to the HED Hazard Identification Assessment Review Committee for further evaluation and possibly revising the toxic endpoint selection for risk assessment. The DERs are attached and executive summaries are as follows.

Acute Neurotoxicity Study in Rats

In an acute neurotoxicity study (MRID 44487501), methomyl (DPX-X1179-512, 98.6% a.i.) was administered by gavage to Crl:CD BR rats (52 rats/dose/sex) at dose levels of 0, 0.25, 0.50, 0.75, or 2.0 mg/kg. An additional 12 rats/sex were assessed to determine baseline neurobehavioral parameters prior to dosing; ten of these animals were then bled and sacrificed in order to establish baseline cholinesterase activity in blood and brain tissues.

No mortality occurred in any of the dose groups in the neurotoxicity subgroup. One female in the clinical pathology subgroup, which had received a 0.75 mg/kg dose, was sacrificed *in extremis* on day 2. Tremors in the 2.0 mg/kg group were noted in both males and females (5/40 of each sex). FOB and MA parameters did not show significant changes compared with controls.

Food consumption was only determined for the neurotoxicity subgroup where values for test animals were found to be comparable to controls. Body weight gain was found to be reduced significantly (73% of the control) in the 2.0 mg/kg female neurotoxicity subgroup during days 2-8. No treatment-related gross or microscopic histopathologic abnormalities were noted.

Brain cholinesterase inhibition was found in all test animals of both sexes receiving a dose ≥ 0.50 mg/kg. Plasma and RBC cholinesterase inhibitions were observed in females at ≥0.50 mg/kg and in Males at ≥ 0.75 mg/kg. By day 2, enzyme activity returned to normal levels for all animals in all dose groups.

The LOAEL is 0.50 mg/kg, based on brain cholinesterase inhibition in all test animals of both sexes, and plasma and RBC cholinesterase inhibition in females receiving this dose. The NOAEL is 0.25 mg/kg. This study is classified Acceptabl/Guideline and satisfies the requirements for an acute neurotoxicity study in rats (81-8).

Subchronic Neurotoxicity Study in Rats

In a subchronic oral neurotoxicity study (MRID 44666201), groups of Crl:CD®BR rats (21/sex/group) were administered Methomyl Technical (Purity 98.6%) in the diet for 13 weeks at dose levels of 0 (control), 20, 50, 150, or 1500 ppm (0, 1.29, 3.14, 9.42, or 94.9 mg/kg/day for males and 0, 1.48, 3.85, 11.2, or 113 mg/kg/day for females, respectively).

Methomyl

There were no substance related mortalities prior to scheduled termination. Clinical signs indicating toxicity were observed among males and females treated with 1500 ppm Methomyl Technical. Males and females treated at this concentration exhibited tremors, males exhibited more aggressive behavior, and females had an increased incidence of alopecia.

Significant treatment-related decreases ($p<0.05$) in body weight (76-80% of controls in males and 81-85% fo controls in females), total body weight gains (64% and 61% of controls for males and females, respectively), food consumption, and food efficiency were observed at 1500 ppm throughout the study.

During the FOB assessment, males and females treated at the highest dose (1500 ppm) were perceived as difficult to handle and exhibited ptosis and abnormal pupillary responses. Females in the highest dose group had decreased incidences of urination and defecation and increased incidences of low arousal and abnormal gait. The assessments of forelimb and hindlimb grip strength revealed a statistically significant decrease among males treated at 1500 ppm compared to the control group during the week 13 evaluation. This parameter remained unaffected among females treated at all concentrations throughout the study and males in lower concentration groups were unaffected. Colburn motor activity was also similar among treated groups compared to the control groups.

No neuropathological endpoints attributable to administration of the test material were observed during the histological examinations of the peripheral or central nervous systems of these animals at any exposure concentration. Brain cholinesterase activity was significantly decreased ($p<0.05$) compared to controls in males (-19% in week 8) and females (-10% in week 4) treated at 1500 ppm. Red blood cell cholinesterase activity was unaffected by the test material, but the plasma cholinesterase activity of males and females assessed during week 8 of the study was decreased (not significantly).

For systemic toxicity, the NOAEL is 150 ppm (9.4 mg/kg/day for males and 11.2 mg/kg/day for females) and the LOAEL is 1500 ppm (94.9 mg/kg/day for males and 113 mg/kg/day for females) based on decreases in body weight, body weight gain, food consumption, and clinical signs of toxicity. For neurotoxic effects, the NOAEL is 150 ppm and the LOAEL is 1500 ppm based on increased incidences of tremors, abnormal pupillary responses, and difficulty in handling in males and females. This neurotoxic LOAEL is further supported by incidences of abnormal gait among females and decreased forelimb and hindlimb grip strength among males. For cholinesterase inhibition, the NOAEL is 150 ppm and the LOAEL is 1500 ppm based on significant decreases of enzyme activity in the brain at the week-4 (females) and week-8 (males) sampling times. This study is classified Acceptable/guideline and satisfied the Subdivision F guideline requirement for a subchronic oral neurotoxicity

EPA Reviewer: Suhair Shallal, Ph.D. S. Shallal, Date 9/1/99
Toxicology Branch I (7509C)
EPA Secondary Reviewer: Yung Yang, Ph.D. Yung G Yang, Date 9/1/99
Toxicology Branch I (7509C)

013770

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Neurotoxicity - Rat
DP BARCODE: D248061 SUBMISSION CODE:S546517
P.C. CODE: 090301 TOX. CHEM. NO.: 549C
CASE NO.: 819319

TEST MATERIAL (PURITY):DPX-X1179-512 (Methomyl Technical), 98.6%

SYNONYMS: DPX-X1179, Methomyl, S-Methyl - [(methylcarbomoyl)oxy]thioacetimidate

CITATION: Mikles, KA, (1998) Methomyl Technical (DPX-X1179-512): Acute Neurotoxicity Study in Rats. E. I. Du Pont de Nemours and Co., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. HL-1998-01080, February 2, 1998. MRID 44487501. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Newark, Delaware.

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 44487501), methomyl (DPX-X1179-512, 98.6% a.i.) was administered by gavage to Crl:CD BR rats (52 rats/dose/sex) at dose levels of 0,0.25, 0.50, 0.75, or 2.0 mg/kg. An additional 12 rats/sex were assessed to determine baseline neurobehavioral parameters prior to dosing; ten of these animals were then bled and sacrificed in order to establish baseline cholinesterase activity in blood and brain tissues.

No mortality occurred in any of the dose groups in the neurotoxicity subgroup. One female in the clinical pathology subgroup, which had received a 0.75 mg/kg dose, was sacrificed *in extremis* on day 2. Tremors in the 2.0 mg/kg group were noted in both males and females (5/40 of each sex). FOB and MA parameters did not show significant changes compared with controls.

Food consumption was only determined for the neurotoxicity subgroup where values for test animals were found to be

comparable to controls. Body weight gain was found to be reduced significantly (73% of the control) in the 2.0 mg/kg female neurotoxicity subgroup during days 2-8. No treatment-related gross or microscopic histopathologic abnormalities were noted.

Brain cholinesterase inhibition was found in all test animals of both sexes receiving a dose ≥ 0.50 mg/kg. Plasma and RBC cholinesterase inhibitions were observed in females at ≥0.50 mg/kg and in Males at ≥ 0.75 mg/kg. By day 2, enzyme activity returned to normal levels for all animals in all dose groups.

The LOAEL is 0.50 mg/kg, based on brain cholinesterase inhibition in all test animals of both sexes, and plasma and RBC cholinesterase inhibition in females receiving this dose. **The NOAEL is 0.25 mg/kg.**

This study is classified acceptable/ guideline and satisfies the requirements for an oral neurotoxicity study (81-8) in rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Methomyl technical (DPX-X1179-512)
   Synonyms: S-methyl N-[(methylcarbamoyl)oxy]thioacetimidate
   Description: white solid
   Lot/Batch #: 22577
   Purity: 98.6% ai.
   CAS #:16752-77-5

2. Vehicle: HPLC grade deionized water

3. Test animals:
   Species: Rat
   Strain: Crl: CD BR
   Age and weight at study initiation: 49-51 days;
   Source: Charles River Breeding Laboratories, Raleigh, NC
   Housing: individually, in suspended, wire-mesh stainless steel cages
   Diet: Purina Certified Rodent Chow #5002 ad libitum
   Water: tap water from United Water Delaware ad libitum
   Environmental conditions: Temperature: 23 ± 1°C
   Humidity:50 ± 10%
   Air changes: Not reported
   Photoperiod:12 hrs. light/12 hrs. dark
   Acclimation period: 6 days

B. STUDY DESIGN:

1. In life dates - start: 9/17/97 end: 11/18/97

2. Animal assignment
   Animals were assigned to the test groups in table 1 via a stratified computer randomization program so that there are no differences in the pretest mean body weights of all groups within a gender.

Table 1- Group Assignments and Dosages*

Group		Dose Level[a] (mg/kg)
Males	Females	
I	II	0
III	IV	0.25
V	VI	0.5
VII	VIII	0.75
IX	X	2.0

[a] Weight/weight concentration of test substance (adjusted for purity).
* This data was extracted from MRID 44487501, p. 18

Table 2- Number of animals in each group and the type of evaluation*

	NB	NP	CP	CP-BLOOD	CP-BRAIN
Baseline	12	0	0	10	0
Test day 1	12[a]	0	10	10[a]	10[a]
Test day 2	0	0	10	10	10
Test day 8	12	0	10[b]	0	0
Test day 15	12	0	10[b]	0	0
Test day 16	0	6	0	0	0

[a]Assessment conducted at approximately 30 minutes post-dosing
[b]animals from days 8 & 15 were not assessed for enzyme activity since levels were found to return to normal after day 2.
NB= Neurobehavioral assessment (FOB and motor activity).
NP = In situ perfusion for neuropathology.
CP-blood = Blood collection for evaluation of cholinesterase in RBC and plasma
CP-brain = Sacrifice and evaluation of cholinesterase activity in whole brain.
*This data was extracted from MRID 44487501, p.19

3. Rationale for dose selection

The report stated that doses selected for this study were based on a reversibility study and a pilot study on neurobehavioral endpoints assessment (no data was submitted). In the reversibility study, methomyl were administered to rats (40/sex/group) at a dose level of 0 or 3 mg/kg. At 30 minutes

post-dose, 17/40 males and 6/40 females at 3 mg/kg exhibited tremors. During the 2, 3, and 4 hours post-dose period, no clinical signs were observed in any rats. Peak effects of cholinesterase inhibition were observed at 30 minutes post-dose. Plasma, RBC, and brain cholinesterase activities were inhibited 27%, 56%, and 46%, respectively in males and 10%, 41%, and 39%, respectively in females at this time point. In the pilot study which was conducted at 1 mg/kg in male and female rats on neurobehavioral endpoints assessment, tremors were observed at 30 minutes post-dose in 4/5 male and 4/5 female rats administered 1 mg/kg methomyl. Other findings noted in the 1 mg/kg rats during the FOB assessment included low posture, abnormal gait, and uncoordinated righting reflex. Based on these studies, dose levels were selected to be 0, 0.25, 0.5, 0.75, and 2 mg/kg.

4. Diet preparation and analysis

Stock solution was prepared in the morning of the day of dosing by mixing appropriate amounts of test substance with the vehicle, water, and was stored at room temperature. Stability was tested after 6 hours and concentration was tested monthly (three times during the study).

Results -
Stability Analysis: 6 hours at room temperature 92-102 % of nominal
Concentration Analysis: 95-97 % of nominal

Nominal (mg/ml)	Mean Measured[a]	Mean % Nominal[b]	Stability % Nominal[c]
0.025	0.0241	96.2	96.4
0.050	0.0475	94.9	92.4
0.075	0.0719	95.8	94.0
0.200	0.1935	96.8	102.0

[a] Concentration verification based on mean result of duplicate aliquots from the original sample
[b] Calculated by taking the mean result of duplicate aliquots from the original sample divided by the nominal
[c] Stability samples held for 6 hours at room temperature

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics -

Food consumption, body weight, body weight gain, foot splay, and grip strength data were analyzed using Bartlett's test which was applied to determine homogeneity of variance between treatments. Since this test was negative for these data, a univariate analysis of variance with Dunnett's test was used to identify which groups if any were significantly different from the control group.

The Functional Observation Battery (FOB) descriptive parameters as well as the clinical incidence data were evaluated using the Cochran Armitage test for trend. When significance was observed, this test was repeated sequentially for decreasing doses until no significance was observed. If there was a lack of fit or if only two groups were to be compared, Fisher's Exact test with a Bonferroni correction was used.

The cholinesterase data were analyzed using the Levene's test for homogeneity of variances and the Spapiro-Wilk test for normality. If these tests were not significant then pairwise comparisons were made using Dunett's test. When the Shapiro-Wilk test was significant, the data were further analyzed using the Kruskal-Wallis test to make pairwise comparisons between the test and control groups.

Motor activity was analyzed using the Shapiro-Wilk test and Levene's test to detect deviations from a normal distribution and equality of variance, respectively. Since the data were normally distributed and there was equality of variance, the data were analyzed using a univariate analysis of variance with dose as a between subjects factor and test day as a repeated measure.

All data were tested at the $p \leq 0.05$ level.

C. METHODS:

1. Observations:

Animals in the neurobehavioral (NB) substudy were inspected once daily for signs of toxicity and mortality. Animals in the clinical pathology (CP) substudy were observed before dosing on Day 1 and 30 minutes after dosing on Day 1, and once on days 2, 8 and 15 on all surviving animals.

2. Body weight

Animals in the NB group were weighed before dosing on day 1, 2, 8, and 15. The animals in the CP group were weighed once immediately before dosing on day 1.

3. Food consumption

Food consumption for each animal in the NB group only was determined and mean daily diet consumption was calculated as g food/kg body weight/day.

4. Functional Observational Battery (FOB)

Only the rats designed for the neurobehavioral testing were evaluated in the FOB test. Rats were observed prior to exposure to establish their baseline FOB parameters. The FOB was performed again on test day 1 at the time of peak signs (approximately 30 minutes post-dosing), and on days 8 and 15. Each rat was evaluated in the three environments: (1) inside the home cage; (2) upon removing from the home cage and while being handled; and (3) in a standard open field. In addition, fore- and hindlimb grip strength was measured by a strain gauge device; hindlimb splay was assessed by inking the hind paws and releasing the rat from a height of 30 cm onto a piece of paper that covers a padded surface; and heel to heel distance was measured from the inked impressions.

5. Motor Activity (MA)

Motor activity sessions were conducted on the same day as

FOB assessments. Rats were individually tested in one of 30 nominally identical, automated activity monitors (Coulbourn®). The infrared monitoring device enables measurement of two dependent variables: duration of movement and number of movements. MA was assessed prior to exposure (baseline) and following the FOB evaluation on test days 1, 8, and 15.

6. Blood Analsis

Blood was collected three times for erythrocyte and plasma cholinesterase activity analysis from 10 animals/dose/sex.

1- the day before dosing
2- 30 minutes after treatment on Day 1
3- the day after treatment on Day 2

Blood was taken from the orbital sinus of each rat while it was under light carbon dioxide anasthesia.

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the brain and other CNS tissues were sectioned and frozen at -70°C for later analysis. Cholinesterase activity was measured on a Boehringer Manneheim/Hitachi 717 clinical chemistry analyser.

II. **RESULTS:**

A. Observations : Mortality and clinical observations are presented in the tables below. There was no methomyl-related mortality observed. Clinical signs of toxicity including tremors, salivation and lacrimation were observed at 2 mg/kg of both sexes.

MALES

Neurotoxicity Substudy:

Group:	I	III	V	VII	IX
Concentration (mg/kg):	0	0.25	0.5	0.75	2
N:	12	12	12	12	12
Mortality:	0	0	0	0	0
Observation					
ALOPECIA	2 (12) [a]	0	0	2(10)	2(7)
DISCHARGE NOSE	0	0	1(2)	1(3)	0
SORE	0	0	1(3)	0	0
STAIN NOSE	0	1(13)	0	0	0
DISCHARGE EYE	0	0	0	0	2 (3)*

Clinical Pathology Substudy:

Group:	I	III	V	VII	IX
Concentration (mg/kg):	0	0.25	0.5	0.75	2
N:	40	40	40	40	40
Mortality:	0	0	0	0	0
Observation					
ALOPECIA	0	1(2)	1 (1)	1 (1)	0
SCAB	0	0	0	1 (1)	0
SORE	0	2(2)	0	1 (1)	0
STAIN CHIN	0	0	1 (1)	1 (1)	1 (1)
STAIN NOSE	0	0	0	2(2)	0
TREMORS	0	0	0	0	5 (1)*

FEMALES

Neurotoxicity Substudy:

Group:	II	IV	VI	VIII	X
Concentration (mg/kg):	0	0.25	0.5	0.75	2
N:	12	12	12	12	12
Mortality:	0	0	0	0	0
Observation					
ALOPECIA	4 (11)[a]	2(3)	3(10)	1(2)	1(14)
DISCHARGE NOSE	0	0	1(2)	0	0
SORE	0	1 (1)	0	0	0

Clinical Pathology Substudy:

Group:	II	IV	VI	VIII	X
Concentration (mg/kg):	0	0.25	0.5	0.75	2
N:	40	40	40	40	40
Mortality:	0	0	0	1	0
Observation					
ALOPECIA	1(1)	0	0	1(1)	0
DISCHARGE NOSE	0	0	1(2)	1(2)[b]	0
DISCHARGE EYE	0	0	0	0	1(1)
ENOPHTHALMUS	0	1(1)	0	0	0
HUNCHED OVER	0	0	0	1(2)	0
MISSHAPEN EAR	1(1)	0	0	1(1)	1(1)
SALIVATION	0	0	0	0	1(1)
SHORT TAIL	0	0	1(1)	0	0
SORE	0	0	1(1)	0	0
SWOLLEN CHEST	0	0	0	1(2)[b]	0
TREMORS	0	0	0	1(1)	5(1)*

[a] Numbers in parentheses indicate the median for days-on-test when the given sign was first observed.

[b] Clinical sign occurred in the rat that was sacrificed in extremis on test day 2.

* Statistically significant difference from control at $p<0.05$

The above data was extracted from MRID 44487501, p. 43-45

B. Body weight gain:

For test days 2-8, females in the 2.0 mg/kg dose group had a significantly lower mean body weight gains than controls. At test day 8-15, their body weight gains recovered to control levels. Food consumption values were comparable with controls for both males and females.

Mean Body Weight Gains of Male Rats (g)

Test Day	Group I 0 mg/kg N=12	Group III 0.25 mg/kg N=12	Group V 0.5 mg/kg N=12	Group VII 0.75 mg/kg N=12	Group IX 2 mg/kg N=12
1-2	6.1 (5.6)[a]	5.4 (4.3)	7.5 (5.2)	7.5 (5.2)	5.6 (5.4)
2-8	51.2 (9.1)	51.9 (10.5)	48.5 (8.5)	43.1 (6.9)	44.7 (8.7)
8-15	45.0 (9.8)	43.5 (10.2)	43.0 (7.7)	41.6 (8.5)	37.5 (8.0)
1-15	102.3 (16.8)	100.9 (19.2)	99.0 (14.5)	92.2 (13.2)	87.8 (16.1)

Mean Body Weight Gains of Female Rats (g)

Test Day	Group 11 0 mg/kg N=12	Group IV 0.25 mg/kg N=12	Group VI 0.5 mg/kg N=12	Group VIII 0.75 mg/kg N=12	Group IX 2 mg/kg N=12
1-2	2.3 (3.3)[a]	3.8 (5.3)	3.6 (5.2)	4.5 (7.4)	4.4 (4.3)
2-8	24.9 (4.9)	20.8 (2.9)	20.5 (7.6)	23.6 (6.3)	18.3 (7.0)*
8-15	21.0 (6.6)	17.8 (7.7)	18.9 (4.4)	19.9 (5.8)	21.6 (3.5)
1-15	48.2 (12.1)	42.4 (9.2)	43.0 (8.7)	48.0 (8.0)	44.2 (8.3)

[a] Standard deviation is presented in parentheses.

* Statistically significant difference from control at $p < 0.05$.

The above data was extracted from MRID 44487501, pp. 48-49.

C. Functional Observational Battery (FOB)

There were no statistically significant or toxicologically remarkable changes in FOB were observed at any dose.

D. Motor Activity

There were no statistically significant or toxicologically important findings in the motor activity test on days 1, 8, and 15.

E. Cholinesterase (ChE) Analysis -

At day 1, inhibitions of plasma, RBC, and brain ChE were observed in females at dsoes ≥ 0.5 mg/kg and in males at doses ≥0.75 mg/kg; brain ChE inhibition was also observed at 0.5 mg/kg in males. By day 2, ChE activity was comparable to controls at all dose levels. Summary of the ChE activity is as follows.

SUMMARY OF MEAN CHOLINESTERASE ACTIVITY IN MALE AND FEMALE RATS

PLASMA CHOLINESTERASE

DOSE	MALES						FEMALES					
	Day 0		Day 1		Day 2		Day 0		Day 1		Day 2	
(mg/kg)	(U/L)	% chg	(U/L)	% chg	(U/L)	% chg	(U/L)	% chg	(U/L)	% chg	(U/L)	% chg
0.00	456		456		450		934		840		699	
0.25	425	93%	380	83%	454	101%	886	95%	749	80%	860	123%
0.50	460	101%	397	87%	441	98%	829	89%	649*	69%	769	110%
0.75	456	100%	349*	77%	433	96%	817	87%	562*	60%	854	122%
2.00	435	95%	263*	58%	442	98%	957	102%	602*	64%	955	137%

RBC CHOLINESTERASE

DOSE	Day 0		Day 1		Day 2		Day 0		Day 1		Day 2	
(mg/kg)	(U/L)	% chg	(U/L)	% chg	(U/L)	% chg	(U/L)	% chg	(U/L)	% chg	(U/L)	% chg
0.00	2026		1716		1928		2046		2426		2146	
0.25	2066	102%	1746	102%	1912	99%	1986	97%	2074	85%	2046	95%
0.50	2062	102%	1634	95%	1848	96%	2036	100%	1826*	75%	1994	93%
0.75	1904	94%	1206*	70%	2240	116%	2250	110%	1492*	62%	2024	94%
2.00	2294	113%	928*	54%	2214	115%	2096	102%	1054*	43%	1988	93%

BRAIN CHOLINESTERASE

DOSE	Day 1		Day 2		Day 1		Day 2	
(mg/kg)	(U/g)	% chg	(U/g)	% chg	(U/g)	% chg	U/g	% chg
0.00	11.68		12.09		12.44		12.17	
0.25	11.00	94%	11.80	98%	11.99	96%	12.19	100%
0.50	9.47*	81%	11.93	99%	9.93*	80%	11.81	97%
0.75	8.71*	75%	12.33	102%	8.73*	70%	11.98	98%
2.00	6.24*	53%	12.20	101%	6.08*	49%	12.03	99%

% chg = percentage of control group mean value

* mean values are significantly different from controls.

U/L = units/liter, U/g = units/gram

The above data was extracted from MRID 44487501, p. 34

F. Sacrifice and Pathology:

1. Gross pathology -

No treatment-related abnormalities were noted.

2. Microscopic pathology -

No treatment-related microscopic neuropathology was noted in the test animals. Axon/myelin degeneration was found in some test animals; however, similar findings were also observed in the control animals.

III. DISCUSSION

Fifty-two animals of each sex were dosed at one of five dosage levels (0., 0.25, 0.5, 0.75 and 2.0 mg/kg) and assessments were made to determine the effect of Methomyl on neurobehavior and neuropathology. Animals were dosed once by gavage then observed, and/or blood samples taken or sacrificed at scheduled intervals and CNS tissue removed. No treatment-related mortalities occured, however, one female was sacrificed *in extremis* at the 0.75 mg/kg dose level. Body weight gain decreased during days 2-8 for females in the 2.0 mg/kg level. By days 8-15, body weight gain was back to normal levels. No histopathologic treatment-related effects were noted. In the clinical pathology group, tremors and lacrimation were noted; these symptoms are consistent with cholinesterase inhibition. Animals at the 0.5 mg/kg dose of both sexes displayed either plasma, RBC, or brain cholinesterase inhibition. The effects were reversible; by day 2 of the study, enzyme levels were back to control levels. **The LOAEL is therefore determined to be 0.5 mg/kg and the NOAEL is 0.25 mg/kg.**

DATA EVALUATION REPORT

013770

METHOMYL TECHNICAL (DPX-X1179-512)

STUDY TYPE: SUBCHRONIC ORAL NEUROTOXICITY - RAT (82-7)

MRID 44666201

Prepared for

Health Effects Division
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U.S. Environmental Protection Agency
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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Neurotoxicity – Rat
OPPTS 870.6200 [§82-7]

DP BARCODE: D253745
P.C. CODE: 090301

SUBMISSION CODE: S557602
TOX. CHEM. NO.: 549C

TEST MATERIAL: Methomyl Technical (DPX-X1179-512)

SYNONYMS: Ethanimidothioic acid, N-[[(methylamino)carbonyl]oxy]-methyl ester, DPX-X1179, DPX-X1179-512, Methomyl, S-methyl N-[(methylcarbamoyl)oxy]thioacetimidate

CITATION: Mikles, K.A., 1998. Methomyl Technical (DPX-X1179-512): Subchronic oral neurotoxicity study in rats. E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P.O. Box 50, Newark, Delaware, 19714-0050. Project ID: DUPONT HL-1998-01639, September 25, 1998, MRID 44666201, Unpublished.

SPONSORS: E.I. du Pont de Nemours and Company, Wilmington, Delaware 19898.

EXECUTIVE SUMMARY: In a subchronic oral neurotoxicity study (MRID 44666201), groups of Crl:CD®BR rats (21/sex/group) were administered Methomyl Technical (Purity 98.6%) in the diet for 13 weeks at dose levels of 0 (control), 20, 50, 150, or 1500 ppm (0, 1.29, 3.14, 9.42, or 94.9 mg/kg/day for males and 0, 1.48, 3.85, 11.2, or 113 mg/kg/day for females, respectively).

There were no substance related mortalities prior to scheduled termination. Clinical signs indicating toxicity were observed among males and females treated with 1500 ppm Methomyl Technical. Males and females treated at this concentration exhibited tremors, males exhibited more aggressive behavior, and females had an increased incidence of alopecia.

Significant treatment-related decreases ($p<0.05$) in body weight (76-80% of controls in males and 81-85% fo controls in females), total body weight gains (64% and 61% of controls for males and females, respectively), food consumption, and food efficiency were observed at 1500 ppm throughout the study.

During the FOB assessment, males and females treated at the highest dose (1500 ppm) were perceived as difficult to handle and exhibited ptosis and abnormal pupillary responses. Females

in the highest dose group had decreased incidences of urination and defecation and increased incidences of low arousal and abnormal gait. The assessments of forelimb and hindlimb grip strength revealed a statistically significant decrease among males treated at 1500 ppm compared to the control group during the week 13 evaluation. This parameter remained unaffected among females treated at all concentrations throughout the study and males in lower concentration groups were unaffected. Colburn motor activity was also similar among treated groups compared to the control groups.

No neuropathological endpoints attributable to administration of the test material were observed during the histological examinations of the peripheral or central nervous systems of these animals at any exposure concentration. Brain cholinesterase activity was significantly decreased ($p<0.05$) compared to controls in males (-19% in week 8) and females (-10% in week 4) treated at 1500 ppm. Red blood cell cholinesterase activity was unaffected by the test material, but the plasma cholinesterase activity of males and females assessed during week 8 of the study was decreased (not significantly).

For systemic toxicity, the NOAEL is 150 ppm (9.4 mg/kg/day for males and 11.2 mg/kg/day for females) and the LOAEL is 1500 ppm (94.9 mg/kg/day for males and 113 mg/kg/day for females) based on decreases in body weight, body weight gain, food consumption, and clinical signs of toxicity. For neurotoxic effects, the NOAEL is 150 ppm and the LOAEL is 1500 ppm based on increased incidences of tremors, abnormal pupillary responses, and difficulty in handling in males and females. This neurotoxic LOAEL is further supported by incidences of abnormal gait among females and decreased forelimb and hindlimb grip strength among males. For cholinesterase inhibition, the NOAEL is 150 ppm and the LOAEL is 1500 ppm based on significant decreases of enzyme activity in the brain at the week-4 (females) and week-8 (males) sampling times.

This study is classified **Acceptable/guideline** and satisfied the Subdivision F guideline requirement for a subchronic oral neurotoxicity study (§82-7) in rats.

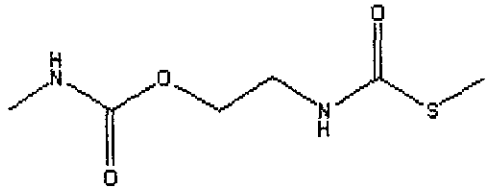
COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Compliance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: Methomyl Technical (DPX-X1179-512)

Description: white solid
CAS No.: 16752-77-5
Lot/Batch No.: not given
Purity: 98.6%
Contaminants: None considered to be of toxicological significance at this time.
Structure:



2. Vehicle

Administered in the diet (Certified Rodent Diet™ #5002 Meal).

3. Test animals

Species: rat
Strain: Crl: CD®BR
Age and mean weight at study initiation: age: 28 days; weight: males: 72.8±8.4 g; females: 72.0 ± 8.0 g
Source: Charles River Laboratories, Inc.
Housing: individually, in suspended wire mesh stainless steel cages
Food: PMI Certified Rodent LabDiet®, 5002. Available *ad libitum*
Water: Tap water, *ad libitum*
Environmental conditions:
Temperature: 23 ± 1 °C
Humidity: 50 ± 10%
Air changes: not given
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 6 days

B. STUDY DESIGN

1. In life dates

Start: January 28, 1998; End: May 1, 1998

2. Animal assignment

The number of animals assigned to the exposure groups is listed in Table 1. Animals were randomly assigned to groups based on body weight, 21 rats/gender were selected for assignment to one of five treatment groups.

Table 1. Study design for rats treated with Methomyl Technical for up to 13 weeks.

Group Male/ Female	Dietary Conc. (ppm)	NB	NP*	CP	Dose (mg/kg/day) Methomyl Technical	
					Males	Females
I/ II	0	12	6	30	0	0
III/IV	20	12	0	30	1.29	1.48
V/VI	50	12	0	30	3.14	3.85
VII/VIII	150	12	0	30	9.42	11.2
IX/X	1500	12	6	30	94.9	113

Data taken from pp. 13and 18, MRID 44666201.
NB, animals designated for neurobehavioral testing
NP, animals designated for neuropathological evaluation
* Subgroup of the NB group
CP, animals designated for cholinesterase evaluation

3. Validation of test methods

The effects of positive control chemicals acrylamide, carbaryl, d-amphetamine, and trimethyl tin (TMT) were evaluated by this laboratory. These data were provided in separate reports which indicated the capability of dectecting effects that may be seen in neurotoxicity studies of this types.

4. Rationale for dose selection

The doses selected for this study were selected by the sponsor after a review of several previously conducted studies. These studies included a 28-day feeding study, four 90-day feeding studies, and two chronic (two-year) feeding studies. Based on these studies, the concentrations for the present study, 0, 20, 50, 150, and 1500 ppm were selected. The lower concentrations of 20 and 50 ppm were selected in anticipation that one of these would be the NOEL and the mid-dose of 150 ppm was selected with the prediction that this dose would produce mild toxic effects. The highest level of 1500 ppm was selected with the intention to produce clear evidence of systemic toxicity.

5. Preparation and analysis of test diet

Methomyl Technical was dissolved in acetone then mixed with the diet at high-speed for six minutes. Control diets were mixed with acetone under the same conditions. Diets were prepared biweekly and refrigerated until used. Diet samples were collected at various intervals during the study for determination of concentration, homogeneity, and stability. Homogeneity of the mixing process was assessed by collecting samples from the top, middle, and bottom of the diet mixer. Stability samples were analyzed after 0, 7, 14, and 21 days at room temperature, and after 21 days of refrigeration. Concentration verification samples were collected from diet buckets.

Results

Homogeneity analysis. Samples of diets containing Methomyl Technical taken from diet preparations were within 8.6 % (C.V.) of the intended concentrations of 20, 50, 150, and 1500 ppm. The mean concentration recovered from samples ranged from 96.5 - 103.6 % of nominal.

Target Concentration. The actual concentrations for samples taken after preparation on days 27, 55, and 83 ranged from 88.7 to 104.0 % of nominal for the 20, 50, 150, and 1500 ppm test diets.

Stability. The stability of Methomyl Technical stored at room temperature (21 °C) for up to 21 days at room temperature were 105.5 and 95.3 % of nominal for the 20 and the 1500 ppm test diets, respectively. After 21 days of refrigeration, the concentrations for the 20 and 1500 ppm diets were 100.5 and 92.0 % of nominal, respectively.

Conclusion. These analyses confirm that the method of diet preparation for this study yields homogeneously mixed diets and that Methomyl Technical is stable in diet preparations at room temperature for > 21 days. The nominal concentrations were representative of actual dietary concentrations fed to the animals.

6. Statistical analysis

Food consumption, body weight, body weight gain, foot splay, and grip strength data were analyzed using Bartlett's test which was applied to determine homogeneity of variance between treatments. Since this test was negative for these data, a univariate analysis of variance with Dunnett's test was used to identify which groups if any were significantly different from the control group.

The Functional Observation Battery (FOB) descriptive parameters as well as the clinical incidence data were evaluated using the Cochran Armitage test for trend. When significance was observed, this test was repeated sequentially for

decreasing doses until no significance was observed. If there was a lack of fit or if only two groups were to be compared, Fisher's Exact test with a Bonferroni correction was used.

The cholinesterase data were analyzed using the Levene's test for homogeneity of variances and the Spapiro-Wilk test for normality. If these tests were not significant then pairwise comparisons were made using Dunett's test. When the Shapiro-Wilk test was significant, the data were further analyzed using the Kruskal-Wallis test to make pairwise comparisons between the test and control groups.

Motor activity was analyzed using the Shapiro-Wilk test and Levene's test to detect deviations from a normal distribution and equality of variance, respectively. Since the data were normally distributed and there was equality of variance, the data were analyzed using a univariate analysis of variance with dose as a between subjects factor and test day as a repeated measure.

No statistical tests were performed on the neuropathology data.

All data were tested at the $p \leq 0.05$ level.

C. METHODS

1. Observations

Cage-side observations for gross signs of substance-related effects were conducted for animals in all groups once daily for the first two weeks and twice daily thereafter. Animals were further observed for mortality and moribundity twice daily throughout the study. At every weighing, rats were individually handled and examined for abnormal behavior and appearance.

2. Body weight

Body weights were determined and recorded once a week during the 13-week study period. Body weights were also recorded on the days of FOB and MA assessment.

3. Food consumption and food efficiency

Food consumption was determined weekly. Food efficiency and mean daily intake of the test substance were calculated from the weekly body weight and food consumption data.

4. Functional observational battery (FOB)

Only the rats designated in the neurobehavioral group were subjected to a FOB prior to exposure and during the 4th, 8th, and 13th weeks of treatment. Rats were divided into four lots and tested over a two-day period with equal representation of each group within each lot.

a. Home Cage Observations

Animals were observed in their closed home cages for posture, palpebral closure, writhing, circling, and biting.

b. Handling observations

Observations during handling from the cage to the open arena were made for ease of removal and handling, fur appearance, and muscle tone. The incidences of vocalizations, piloerection, bite marks on tail/paws, palpebral closure, lacrimation, exopthalmus, and salivation were also recorded.

c. Open arena observations

Animals were observed in an open-arena with the following parameters recorded: unusual responses in activity level, coordination, locomotion, righting reflex, convulsions/tremors, grooming, labored breathing, vocalizations, gait, defecation, and urination.

d. Sensorimotor Tests/Reflexes

When the animals were removed from the open field, they were subjected to the following sensorimotor or reflex tests: approach response, touch response, sharp auditory stimulus, tail pinch, pupil response, grip strength (forelimb and hindlimb), and landing foot splay.

5. Motor activity (MA)

Motor activity measurements were assessed for only those animals designated in the neurobehavioral group. Assessments were conducted following the FOB observations, prior to exposure and during the 4th, 8th, and 13th, weeks of treatment. Individual activity was monitored with an Infrared Motion Activity System. MA was measured for 1 hour.

6. Sacrifice/necropsy/neurohistopathology

At study termination, the neuropathology subgroup animals (6/sex/group) were anesthetized with an overdose of pentobarbital (i.p.) followed by exanguination and whole body perfusion. All animals were examined grossly for lesions when

sacrificed. The brain, spinal cord, and nerves, listed in the table below from the control and high concentration groups, were examined histologically. Peripheral nerve samples were embedded in glycol methacrylate, and stained with hematoxylin and eosin.. The brain, spinal cord, and skeletal muscle were embedded in paraffin, then sectioned and stained with hematoxylin and eosin. An additional set of brain and spinal cord sections were stained with Luxol fast blue and periodic acid Schiff (LFB/PAS).

X	Brain*	X	Spinal Cord	X	Peripheral nerves
X	Forebrain	X	Cervical	X	Sciatic nerve
X	Cerebrum	X	Dorsal and ventral root	X	Tibial nerve
X	Midbrain		fibers	X	Sural nerve
X	Pons	X	Lumbar		
X	Medulla	X	Dorsal and ventral root		
X	Cerebellum		fibers	X	Other
				X	Gastrocnemius muscle
					Gasserian ganglion

* Organs that were also weighed

7. Clinical Chemistry

Blood was collected from animals in the Clinical Chemistry group for measurement of plasma and RBC cholinesterase activity prior to the administration of test diets, and after 4, 8, and 13 weeks of treatment. Rats were fasted prior to the sampling and blood was collected from the orbital sinus while the rats were under light anesthesia. At the 4, 8, and 13 week collections, 10 rats were sacrificed after the collections by decapitation and the brains were removed, stored on ice until frozen at approx. -70 C, for determination of whole brain cholinesterase activity.

Blood samples were stored on ice until anaylzed. RBCs were diluted just prior to analysis. Cholinesterase activities were measured by a colorimetric method (Ellman) on a Hitachi 717 clinical chemistry analyzer using Boehringer Mannheim reagents.

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

There were no mortalities that were attributed to administration of Methomyl Technical in this study. There were two deaths among males treated at low to intermediate doses. One male in the 50 ppm group was found dead on test day 31 (unknown cause), and one male in the 20 ppm group was sacrificed *in extremis* on test day 89 due to an injury. Neither of these deaths were considered to be test substance-related.

Tremors were observed among males and females treated at the high-dose (1500 ppm). During test days 0-27, 32/42 males were affected, and during test days 0-28, 23/42 females at this dose were affected ($p < 0.05$). The incidence declined and after day 55, no animals were observed with tremors. Males treated at 1500 ppm exhibited statistically significantly increased incidences of aggressive behavior and hyperreactivity. Females in the 1500 ppm were observed to have significantly increased incidences of alopecia. Females treated at this concentration also had an increased incidence of colored discharge from the eyes.

Male and female rats treated at lower concentrations (20, 50, and 150 ppm) did not exhibit overt clinical signs of toxicity during the daily observations.

B. BODY WEIGHT AND BODY WEIGHT GAINS

Males and females treated at the high-dose (1500 ppm) were observed to have significantly lower mean body weights at every weighing from test day 7 through test day 91 (Table 2). On day 91, the body weights of high-dose male and female rats were decreased 24% and 19%, respectively compared to the control.

There were no effects on mean body weight among males and females treated at 150 ppm and lower.

Body weight gains among males and females receiving 1500 ppm of the test substance were significantly decreased compared to controls for the overall day 0-91 period. These groups had mean body weight gains that were significantly lower than controls for several intervals during the study as well (Table 2).

No effect on mean body weight gain was observed in females at 50 ppm or lower. Male rats treated at 50 and 150 ppm had body weight gains that were significantly decreased compared the controls for one interval during the study (Table 2). The overall mean body weight gains for these animals were similar to controls.

Table 2. Mean body weights (g) and body weight gains (g) for male and female rats treated with Methomyl Technical for 13 weeks in the diet.

Day	Group/dose (mg/kg)									
	0	20	50	150	1500	0	20	50	150	1500
	Males					Females				
0	185	184	185	183	182	148	148	149	148	147
7 (% cont)	236	236	238	232	181* (77%)	172	171	170	168	139* (81%)
21 (% cont)	327	329	328	320	261* (80%)	213	210	211	208	181* (85%)
35 (% cont)	387	389	389	384	310* (80%)	242	238	239	235	205* (85%)
49 (% cont)	436	439	437	434	344* (79%)	260	255	256	251	218* (84%)
63 (% cont)	465	473	468	468	362* (78%)	273	269	263	267	231* (85%)
77 (% cont)	496	502	496	495	380* (77%)	282	279	275	279	240* (85%)
91 (% cont)	520	508	506	518	395* (76%)	293	286	275	276	237* (81%)
Day	**Weight gains**									
0-7	52	52	52	50	-1.7*	24	23	21	20*	-8*
14-21	41	42	42	40	36*	18	19	18	17	17
42-49	24	26	27	25	17*	11	9	8	7*	6*
63-70	18	15	13*	11*	8*	2	4	5	5	4
84-91	9	6	-8	9	9	5	0.7	2	-2	1
0-91 (% cont)	340	332 (98%)	317 (93%)	330 (97%)	218* (64%)	146	134 (93%)	129 (88%)	130 (89%)	89* (61%)

Data taken from pp. 60-67 MRID 44666201.
*$p<0.05$

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Mean food consumption values for the entire study are presented in Table 3. Males and females treated with 1500 ppm of the test substance had significantly lower food consumption compared to controls for the overall day 0-91 treatment period. Males treated at this concentration had consistently decreased food consumption while females had a period of normal food consumption during the middle of the study (Table 3).

Males and females treated at lower concentrations did not exhibit decreased food consumption at any time during the study.

Table 3. Mean food consumption (g) values for male and female rats treated with Methomyl Technical for 13 weeks in the diet.

Day	Group/dosage (ppm)									
	Males					Females				
	0	20	50	150	1500	0	20	50	150	1500
0-7 (% cont)	22.4	22.2	22.3	21.5	11.1* (50%)	17.1	16.4	16.7	16.1	8.7* (51%)
0-28	24.4	24.3	24.3	23.6	18.4*	17.5	17.2	17.8	17.4	14.1*
28-56	25.1	25.7	25.3	25.3	20.7*	18.5	18.2	18.3	18.2	17.0
56-91	25.9	26.1	25.7	25.8	20.6*	18.0	18.2	18.2	17.2	16.0*
0-91 (% cont)	25.2	25.2	25.4	25.1	19.7* (78%)	18.3	18.3	17.9	17.8	15.4* (84%)

Data taken from p. 68-71, MRID 44666201.
*p <0.05

Table 4. Mean food efficiencies (g weight gain/g food consumed) for male and female rats treated with Methomyl Technical in the diet for 13 weeks.

Day	Group/dosage (ppm)									
	Males					Females				
	0	20	50	150	1500	0	20	50	150	1500
0-7	0.328	0.332	0.332	0.329	-0.053*	0.201	0.202	0.180	0.180	-0.166*
0-28	0.259	0.263	0.257	0.260	0.202*	0.156	0.158	0.150	0.155	0.117*
28-56	0.123	0.127	0.127	0.131	0.114	0.068	0.070	0.067	0.068	0.067
56-91	0.075	0.070	0.055	0.068	0.063	0.035	0.026	0.033	0.029	0.027
0-91 (% cont)	0.148	0.144	0.138	0.144	0.122* (82%)	0.087	0.080	0.079	0.080	0.064* (74%)

Data taken from p. 72-75, MRID 44666201.
* p< 0.05

Male and female rats exposed to 1500 ppm had significantly decreased food efficiency values for the overall day 0-91 period (Table 4). Animals treated at lower concentrations were unaffected by the test material in this respect. A few spurious decreases were observed for some intervals at lower treatment doses.

D. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Grip Strength

Statistically significant decreases in mean hindlimb and forelimb grip strength compared to the control animals (29% and 22%, respectively) were observed in the 1500 ppm males on week 13. No test substance-related or statistically significant differences in mean forelimb or hindlimb grip strength were observed for females in the 1500 ppm group or in any of the lower male concentration groups compared to the controls.

Foot Splay

There were no observations of toxicologically significant differences in mean hindlimb splay between treated and control groups during any of the FOB assessment sessions. Females treated at 50 ppm had significantly decreased foot splay only during the week-13 FOB assessment.

Other FOB Endpoints

Several clinically relevant observations were made among males and females receiving 1500 ppm of the test substance. The incidences of these observations were significant ($p<0.05$) and included difficulty removing the rats from their cages, difficulty handling the rats, ptosis, and abnormal pupillary responses. Additionally, females treated at 1500 ppm had significantly increased incidences of low arousal and abnormal gait compared to the control group. Spurious increases and decreases in urination and decreases in defecation were also observed for females in the 1500 ppm group.

E. MOTOR ACTIVITY

Colburn motor activity was not affected by Methomyl Technical administration. There were some significant differences between treated groups compared to the controls for various ten minute blocks, these were considered spurious findings. Females in the 1500 ppm group had a significant decrease in total number of movements compared to the control group during the week-13 assessment. This finding was minimized by the observation that the control group had an abnormally increased total number of movements compared to the historical control data.

F. NEUROPATHOLOGY

Neuropathological examination revealed no findings that were attributable to the administration of Methomyl Technical. Incidental findings were observed with the same frequency in the control and treated groups.

G. CHOLINESTERASE ACTIVITY

Brain

Male and female rats that were administered 1500 ppm test substance exhibited minimal to mild brain cholinesterase inhibition (Table 5). Males treated with 1500 ppm had significantly ($p < 0.05$) decreased enzyme activity at the week-8 sacrifice. The decrease in enzyme activity was 81% of the control group mean at this time point. At the week-4 and week-13 sampling times, the mean brain cholinesterase activity for males treated with 1500 ppm were 92 % and 95 % of the control group mean, respectively. These decreases were not statistically significant.

Among females treated at 1500 ppm, the most pronounced decrease in brain cholinesterase activity was observed at the week-4 sampling time point when measured activity was 90 % of the control mean ($p < 0.05$). The measured mean cholinesterase values for females in this concentration group at the week-8 and week-13 sampling times were 96 and 97% of control values, respectively.

Table 5. Mean brain cholinesterase (U/g) findings in male and female rats fed Methomyl Technical for up to 13 weeks in the diet.

Concentration	Pretest	Week 4	Week 8	Week 13
Males				
0	NM	11.29	11.24	10.65
20	NM	11.29(100%)	10.90(97%)	10.37(97%)
50	NM	11.28(100%)	10.98(98%)	10.53(99%)
150	NM	11.35(101%)	10.82(96%)	10.81(102%)
1500	NM	10.41(92%)	9.14*(81%)	10.09(95%)
Females				
0	NM	11.95	11.57	11.15
20	NM	11.49(96%)	11.18(97%)	10.72(96%)
50	NM	11.65(97%)	11.36(98%)	10.86(97%)
150	NM	11.24(94%)	11.61(100%)	10.71(96%)
1500	NM	10.79*(90%)	11.15(96%)	10.80(97%)

Data taken from pp. 41 and 42, MRID 44666201. *$p < 0.05$

Red Blood Cell

There was no evidence of cholinesterase inhibition among male or female rats treated

with Methomyl Technical at any dose level in this study.

Table 6. Mean RBC cholinesterase (U/L) findings in male and female rats fed Methomyl Technical for up to 13 weeks in the diet.

Concentration	Pretest	Week 4	Week 8	Week 13
Males				
0	2084	1790	1494	1514
20	2172(104%)	1838(103%)	1478(99%)	1688(111%)
50	2036(98%)	1360(76%)	1610(108%)	1684(111%)
150	2302(110%)	1734(97%)	1872(125%)	1520(100%)
1500	1934(93%)	1992(111%)	1652(111%)	2118*(140%)
Females				
0	2090	1834	1718	2228
20	2046(98%)	1860(101%)	1546(90%)	2006(90%)
50	1706(83%)	1568(85%)	1728(101%)	1756(79%)
150	1892(91%)	1780(97%)	1762(103%)	1784(80%)
1500	1906(91%)	1974(108%)	2336(136%)	2332*(105%)

Data taken from pp. 41 and 42, MRID 44666201. *$p < 0.05$

Plasma

Plasma cholinesterase activity among male rats treated with 1500 ppm of the test material was mildly at the week-8 sampling decreased compared to the control group. Females treated at this dose had cholinesterase activities that were 92, 89, and 89% of control values for the week-4, -8, and -13 sampling times, respectively. These values were not significantly decreased compared to the control. Two male rats had cholinesterase activity that was 65% of the control mean at the week-8 sampling time.

Table 7. Mean plasma cholinesterase (U/L) findings in male and female rats fed Methomyl Technical for up to 13 weeks in the diet.

Concentration	Pretest	Week 4	Week 8	Week 13
Males				
0	448.8	364	384.7	425.7
20	493.8(110%)	414.6(114%)	428.2(111%)	399.7(94%)
50	486.3(108%)	399.6(110%)	395.7(103%)	427.3(100%)
150	484.6(108%)	410.2(113%)	397.0(103%)	413.2(97%)
1500	478.2(107%)	406.6(112%)	353.9(92%)	440.1(103%)
Females				
0	551.1	1379.5	1697.4	2009.7
20	584.8(106%)	1476.2(107%)	2059.8(121%)	1869.2(93%)
50	554.7(101%)	1404.6(102%)	2205.6(130%)	2305.3(115%)
150	519.2(94%)	1263.8(92%)	1908.0(112%)	1967.9(98%)
1500	592.7(108%)	1273.8(92%)	1504.3(89%)	1796.5(89%)

Data taken from pp. 41 and 42, MRID 44666201. *p < 0.05

III. DISCUSSION

A. DISCUSSION

There were no substance-related mortalities in this study. One male rat in the 50 ppm group was found dead on test day 31 and a gross necropsy failed to reveal the cause of death. This death was not attributed to test substance administration due to the absence of any other signs of toxicity at this treatment level and an absence of mortalities at higher doses. Another male treated at 20 ppm was sacrificed *in extremis* due to an injury, this mortality was clearly not related to consumption of the test material.

Daily observations for clinical signs revealed several test substance-related abnormalities among males and females in the highest dose group (1500 ppm). Males and females treated at this dose exhibited tremors quite frequently throughout the first half of the study period, this effect was not observed after day 55. Males treated with 1500 ppm were observed to have significantly increased incidences of aggressive behavior and hyperreactivity. These observations are indicative of a neurotoxic effect at 1500 ppm.

Significant treatment-related decreases ($p<0.05$) in body weight (76-80% of controls in males and 81-85% fo controls in females) and total body weight gains (64% and 61% of

controls for males and females, respectively) were observed in rats exposed to 1500 ppm Methomyl Technical. The mean body weights and body weight gains of males and females treated at this dose were significantly decreased compared to the control group throughout the study. Since these decreases were ≥20% compared to the respective control groups throughout much of the study period, this observation clearly indicates systemic toxicity at this dose. A few spurious decreases in body weight and body weight gain were observed in the lower dose groups compared to the controls, however these incidences did not seem to be dose-related and were infrequent.

Food consumption (78% and 84% of controls for males and females, respectively) and food efficiency (82% and 74% of controls for males and females, respectively) were significantly decreased among males and females treated with 1500 ppm of Methomyl Technical in the diet for the overall interval, day 0-91, and during several intervals throughout the study. This effect was clearly treatment-related.

Several neurotoxic endpoints were observed during the FOB assessments in this study that were regarded as treatment-related. These observations were confined to the highest dose group. Males and females treated at the highest dose (1500) were perceived as difficult to handle and had abnormal pupillary responses. Additionally, females treated at 1500 ppm had significantly increased incidences of low arousal and abnormal gait compared to the control group. The assessments of forelimb and hindlimb grip strength as well as hindlimb splay were significantly decreased among males in the 1500 ppm treatment group compared to the control. No differences for any of the female treated groups compared to the control groups were observed as well as among males treated at lower doses. These observations support a LOAEL for neurotoxic effects at 1500 ppm.

Colburn motor activity was not affected by the administration of the test substance. Females in the 1500 ppm group had a significant decrease in total number of movements compared to the control group at the week-13 assessment. However, this finding was minimized by the observation that the control group had an abnormally increased total number of movements compared to the historical control data.

Neurohistopathology examinations did not indicate exposure-related effects. Observations of abnormalities among treated animals occurred with the same incidence in the control groups and were considered common findings for this species and strain.

A significant inhibition of brain cholinesterase activity was observed among males and females in the 1500 ppm treatment groups in this study. Although the significant findings occurred at different sampling times for males and females, the inhibition was approximately 20% and statistical significance was obtained. The decreased enzyme activity in this study is attributable to the administration of the test material. There was only a slight (not statistically significant) inhibition of plasma cholinesterase at this dose and red blood cell enzyme activity remained unaffected throughout the study.

The NOAEL for systemic toxicity is 150 ppm (9.4 mg/kg/day for males and 11.2 mg/kg/day for females). The LOAEL is 1500 ppm (94.9 mg/kg/day for males and 113 mg/kg/day for females) based on the substance-related effects on body weight, body weight gain, food consumption, and clinical signs of toxicity. The NOAEL for neurotoxic effects is 150 ppm (9.4 mg/kg/day for males and 11.2 mg/kg/day for females). The neurotoxicity LOAEL is 1500 ppm (94.9 mg/kg/day for males and 113 mg/kg/day for females) based on the incidence of tremors, abnormal pupillary responses, and difficulty in handling among males and females. This LOAEL is further supported by incidences of abnormal gait among females and decreased forelimb and hindlimb grip strength among males. The NOAEL for decreased cholinesterase activity is 150 ppm (9.4 mg/kg/day for males and 11.2 mg/kg/day for females). The LOAEL for decreased cholinesterase activity is 1500 ppm (94.9 mg/kg/day for males and 113 mg/kg/day for females) based on significantly decreased enzyme activity in the brain at the week-4 (females) and week-8 (males) sampling times.

B. STUDY DEFICIENCIES

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None.